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Circulating Inflammatory-associated Proteins in the First Month of Life and Cognitive Impairment at Age 10 years in Children Born Extremely Preterm

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Abstract

Objectives—To evaluate whether in children born extremely preterm, indicators of sustained systemic inflammation in the first month of life are associated with cognitive impairment at school age.

Study design—873 of 966 eligible children previously enrolled in the multicenter Extremely Low Gestational Age Newborn Study from 2002–2004 were evaluated at age 10 years. We

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The other authors declare no conflicts of interest.

analyzed the relationship between elevated blood concentrations of inflammation-associated proteins in the first 2 weeks (“early elevations”; n=812) and the 3rd and 4th week (“late elevations”; n=532) of life with neurocognition.

Results—Early elevations of CRP, TNF-alpha, IL-8, ICAM-1, and EPO were associated with IQ values >2 SD below the expected mean (ORs: 2.0–2.3) and with moderate to severe cognitive impairment on a composite measure of IQ and executive function (ORs: 2.1–3.6). Additionally, severe cognitive impairment was associated with late protein elevations of CRP (OR:4.0; 95% CI 1.5, 10), IL-8 (OR:5.0; 1.9, 13), ICAM-1 (OR:6.5; 2.6, 16), VEGF-R2 (OR:3.2; 1.2, 8.3), and TSH (OR:3.1; 1.3, 7.3). Moderate cognitive impairment was most strongly associated with elevations of IL-8, ICAM-1, and VEGF-R2. When four or more inflammatory proteins were elevated early, the risk of having an IQ<70 and having overall impaired cognitive ability was more than doubled (ORs:2.1–2.4); the presence of four or more inflammatory protein elevated late was strongly linked to adverse cognitive outcomes (ORs:2.9–4.8).

Conclusion—EP children who had sustained elevations of inflammation-related proteins in the first postnatal month are more likely than EP peers without such elevations to have cognitive impairment at 10 years.

Keywords

extremely preterm infants; inflammation-related proteins; cognition; school age

Advances in neonatal intensive care have increased the survival of extremely preterm children born before 28 weeks of gestation.[1] Little progress has been made, however, in the prevention of moderate to severe neurocognitive impairments that affect about 40% of EP survivors.[1–13] Improvement in outcomes of EP children requires a better understanding of the antecedents and causes of neurocognitive impairment in this population, which could lead to development of new technologies and approaches. In the initial phase of the Extremely Low Gestational Age Newborn (ELGAN) Study that evaluated more than 900 children born before 28 weeks gestation, neonatal elevations of specific molecular biomarkers (ie, inflammation-associated proteins in blood), robustly predicted cognitive impairment at 2 years of age.[14, 15] Moreover, concentrations of inflammation-related proteins in blood spot samples collected in the 3rd and 4th weeks of life were associated with two-year outcomes beyond associations with protein concentrations from the first two postnatal weeks alone.[16]

Cognitive assessments at 2 years among infants born with extremely low birth weight, however, have correlated only modestly with school-age cognitive abilities[12, 17], which better predict later academic achievement and vocational and social competence.[18] For a more definitive evaluation of the long-term impact of neonatal elevations of inflammation-related proteins, we assessed cognitive abilities during school age in the ELGAN Study cohort when assessment of cognitive ability is reliable.

We report here analyses that test the hypothesis that persistent, elevated concentrations of circulating inflammation-associated proteins in the first 2 postnatal weeks (early) are associated with an increased risk of cognitive deficits at 10 years of age in EP children. We

also test the hypothesis that persistent, elevated concentrations of circulating inflammation-associated proteins in the 3rd and 4th postnatal weeks are associated with risk of cognitive deficits at age 10 years beyond the risk conferred by the "early" elevations of inflammatory proteins.

Methods

The ELGAN Study is a multicenter observational study of the risk of structural and functional neurologic disorders in EP infants. During the years 2002–2004, women delivering before 28 weeks gestation were asked to enroll in the study. A total of 1249 mothers of 1506 infants consented to participate. At 10 years of age, 966 surviving children for whom we obtained neonatal blood specimens for measurement of inflammation-related proteins, were targeted for recruitment. The families of 889 (92%) of these children returned for follow up. The institutional review boards of all participating institutions approved enrollment and consent procedures for this follow-up study.

Of the 889 children evaluated, 11 did not accompany the parent or caregiver during the follow-up visit (hence informed consent could not be obtained), and 5 children did not cooperate with the child assessment, leaving a final sample of 873 children. In the analyses that included only early protein elevations, of the 873 participants, we evaluated risk in the 812 for whom both early blood samples and 10-year outcome data were available. When we evaluated risk associated with late elevations (3rd and 4th postnatal week), we considered the risk above that attributable to the early elevations, and included only the 532 children for whom we possessed blood samples at both the early and late time intervals. Because of severe motor, visual and cognitive disability, 29 children were assigned floor scores on all tests, and 11 were assigned floor scores on some tests.

Families willing to participate were scheduled for one visit, usually at the institution of birth. Child measures were selected to provide the most comprehensive assessment of cognitive and academic function obtainable in a single testing session. Evaluations were administered by certified child psychologists blinded to clinical information in a 3 to 4 hour session that included breaks. All psychologist examiners underwent a 1-day in-person training and verification of competency for administering the neurocognitive test battery.

Assessments

General cognitive ability (or IQ) was assessed with the School-Age Differential Ability Scales–II (DAS-II[19], and Verbal and Nonverbal Reasoning scales. Because DAS-II Verbal and Nonverbal IQ scores were strongly correlated within the sample, the mean of these two meaures was used as an estimate of general cognitive ability.

Attention and executive function were assessed with the DAS-II and the NEPSY-II.[20]. DAS-II Recall of Digits Backward and Recall of Sequential Order measured verbal working memory. NEPSY-II Auditory Attention and Auditory Response Set measured sustained auditory attention, set switching and inhibition. NEPSY-II Inhibition-Inhibition and Inhibition-Switching tasks measured simple inhibition and inhibition in the context of set shifting, respectively. NEPSY-II Animal Sorting measured concept generation and mental

flexibility. For purposes of these analyses, executive function was considered in conjunction with IQ using a Latent Profile Analysis construct.

We used latent profile analysis (LPA) to classify children in our sample into subgroups based on similarities in their profiles of IQ and EF scores. These analyses identified four subgroups in our cohort corresponding to overall cognitive functioning that was normal (34% of cohort, with mean IQ and EF scores within normal range on all measures), low-normal (41%, with mean IQ and EF scores ranging from 0.5 to 1 standard deviation below the norm), moderately impaired (17%, with mean IQ and EF measures between 1.5 and 2.5 standard deviations below the norm), and severely impaired (8%, with mean IQ and EF measures 3 to 4 standard deviations below the norm).

Blood Protein measurements

Drops of whole blood were collected on (Schleicher & Schuell 903) filter paper on the first postnatal day (range: 1–3 days) and the 7th (range: 5–8 days) and 14th (range: 12–15 days) postnatal days. Twenty-eight proteins taken from blood samples in the first two weeks of life and 16 proteins taken from blood sample in the 3rd and 4th weeks of life were measured in the Laboratory of Genital Tract Biology, Brigham and Women's Hospital, using the Meso Scale Discovery multiplex platform and Sector Imager 2400 (Meso Scale Discovery, Gaithersburg, MD), which has been validated against ELISA. Details about the procedure for processing the blood spots and for measuring protein concentrations and absolute value ranges for 28 inflammation-regulating proteins are explained elsewhere.[21]

In the blood spot samples we considered for analyses the 16 proteins that were measured at all 5 points of time in the first month of life, including cytokines: Interleukin (IL)-1 β (IL-1β), Interleukin-6 (IL-6) and its receptor (IL-6R), tumor necrosis factor-alpha (TNF-α) and one of its receptors (TNF-alpha R2); adhesion molecules: intercellular adhesion molecule-1 (ICAM-1); growth factors: vascular endothelial growth factor (VEGF) and one of its receptors (VEGF-R2); chemokines: Interleukin-8 (IL-8), regulated upon activation, and normal T-lymphocyte expressed, and (presumably) secreted (RANTES); metalloproteinases: matrix metalloproteinase-9 (MMP-9); liver-produced: serum amyloid A (SAA), C-reactive protein (CRP); endocrine regulators: thyroid-stimulating hormone (TSH) and erythropoietin (EPO), and neutrophil- derived effectors: myeloperoxidase (MPO).

Volumes of blood spotted on the filter paper varied, and to standardize the inflammation protein measurements we eluted each fixed spot area in the same volume of elution buffer and normalized the concentration of each biomarker to total protein concentration.[22] Measurements were made in duplicate, and the mean served as the basis for all tables and analyses. The protein concentrations varied with gestational age, and with the postnatal day of collection.[23, 24] In addition, measurements from the first two weeks of life were assayed in 2009–2010 whereas the proteins obtained from weeks 3 and 4 of life were assayed in 2015, and, although the distributions of each were similar, they were not identical. Consequently, we divided our sample into 30 groups defined by gestational age category (23–24, 25–26, 27 weeks), postnatal day of blood collection (1, 7, 14, 21 and 28), and measurement set (2009–2010, 2015). Four considerations prompted us to operationalize using highest quartile protein values for a particular gestational age and postnatal day/week

of sampling as a measure of inflammation signal. First, normative data for circulating inflammation-associated proteins in extremely low gestational age newborns (ELGANs) are not available. Second, circulating protein concentrations in our cohort of ELGANs varied according to gestational age and by postnatal day or week the blood was sampled. Third, the protein values did not conform to a normal distribution. Finally, because weeks 3 and 4 blood measures were completed nearly 10 years apart, use of quartile minimizes potential impact of protein degradation over time. Sustained elevation for a particular protein was defined as a protein concentration in the highest quartile on at least 2 of the 3 measures obtained in the first 2 weeks and in the highest quartile on both measures for the 3rd and 4th week samples.

Data analyses

We evaluated two measures of cognition using IQ and LPA categories. We use cognitive impairment as an overarching term that refers to either having i*mpaired IQ* (mean verbal and nonverbal IQ less than 70) or having *impaired overall cognitive functioning* (moderately or severely impaired groups based on LPA, which integrates measures of IQ and executive function abilities). We also present 2 intermediary categories based on IQ z-scores between −2 and −1 or being in the low normal group based on LPA. We tested the hypothesis that infants with sustained inflammation were not more likely than infants without sustained inflammation to have cognitive impairment at age 10 years. We defined cognitive impairment as IQ at least 2 Z-scores below normative expectation or as moderately or severely impaired by LPA.

In the analyses in which we considered the number of inflammation-related protein elevations as a predictor of outcome, we used a subset of 9 proteins (CRP, SAA, IL-1β, IL-6, TNF-α, IL-8, ICAM-1, MMP-9, VEGF) common to both the early and late protein sets. These proteins have been associated consistently with structural and functional neurological outcomes in the literature and in previous ELGAN Study analyses.[14, 25, 26]

Logistic regression and multinomial logistic regression models were used to examine the association between measures of inflammation and cognitive impairment, controlling for public insurance status at birth (as a measure of low SES) and child sex. First, we explored whether sustained elevation of specific, individual proteins in the first two postnatal weeks of life (early) predicted cognitive impairment. Second, we explored whether breadth of early inflammation (with high defined by the presence of sustained elevations of 4 or more inflammation-related proteins and moderate inflammation defined by sustained elevations in 2–3 proteins) was associated with cognitive impairment. Finally, using time-oriented models, we evaluated the added contribution to risk of cognitive impairment from protein elevations in the 3rd and 4th weeks of life, controlling for early protein elevations.[27]

Odds ratios and 95% Confidence Intervals were used to describe associations between markers of inflammation and our 3 category IQ outcome (IQ z-score −2 or below, between −2 and −2, or above −1) and 4 category overall cognitive functioning outcome (severely impaired, moderately impaired, low normal function, normal cognitive function). Odds ratios are given for each impairment category relative to the highest functioning reference category

Results

Approximately 20% of the cohort was born at 23–24 weeks, 45% were born at 25–26 weeks, and 34% were born at 27 weeks gestation. Seven percent (38/532) were born small for gestational age, and 27% (132/487) had a MDI score <70 at 2 years of age. Impaired MDI values were highly associated with impaired IQ and with non-normal overall cognitive impairment at age 10 years. Children whose mothers had indicators of low socioeconomic status (less than high school education, single status, and public insurance), who were non-white, and who were male were more likely to have cognitive impairment (Table I).

In the first two weeks, elevated concentration of CRP, TNF-alpha, IL-8, ICAM-1, and EPO individually was associated with Impaired IQ (ORs ranging from 2.0 to 2.3). (Table II) These proteins, as well as CRP and SAA, also were associated with moderately and severely impaired overall cognitive functioning (ORs: 2.1 to 3.6 for the severly impaired group and from 2.0 to 2.4 for the moderately impaired group) (Table III). After adjusting for early protein elevations, late elevations of CRP, VEGF-R2, and TSH were associated with severe impairment (ORs ranged from 3.1 to 6.5). IL-8, ICAM-1, and VEGF-R2 also were significantly higher in the groupts testing as moderately impaired and low normal compared with groups testing as normal.

The presence of ≥4 elevations of inflammatory protein was associated with impaired IQ (OR: 2.1 to 2.4) (Table IV). Additionally, elevations of ≥4 proteins were associated with moderately impaired overall cognitive function (OR: 2.8; 95% CI 1.5, 5.0) and low normal overall cognitive function (OR: 1.7; 95% CI 1.01, 2.7).

After adjusting for early elevations of protein in the 532 children for whom we assayed proteins both in the early and late periods (Table IV), late elevation of ≥4 proteins was associated with impaired IQ, severe and moderate overall cognitive impairment, (ORs ranged from 2.9 to 5.1).

Among the proteins evaluated, the two most consistently associated with cognitive impairment were IL-8 and ICAM-1. Early elevations of these were associated with severly decreased IQ (IL-8-OR: 2.3;1.4,3.8; ICAM-1- OR: 2.3; 1.4,3.7) (Table II). When adjusting for early elevations of proteins, late elevations of these two proteins were associated with severe overall cognitive impairment (IL8- OR: 5.0; 1.9,13; ICAM-1- OR: 6.5;2.6,16) (Table III) . Both of these protein elevations also were associated with moderately impaired and low normal overall impairment from the LPA grouping with ORs that ranged from 2.7 to 6.3.

Discussion

Persisting, elevated concentrations of a broad number of circulating inflammation-associated proteins in the first 4 postnatal weeks were associated with cognitive impairment at age 10 years. The association was found for IQ measures, and a categorical outcome that summarized IQ and executive function. Additionally, IL-8 and ICAM-1 were the circulating proteins in the first month of life most strongly associated with cognitive impairment at 10 years of age.

Previously, we demonstrated that persisting, elevated levels of circulating inflammation-associated proteins in the first 2 postnatal weeks of life were associated with cognitive impairment at 2 years of age in the ELGAN Study cohort.[14, 15] Now we provide evidence that elevation of circulating inflammation-associated proteins detectable in the 3rd and 4th postnatal weeks contribute to risk of cognitive impairment beyond the risk posed by the presence of such proteins in the first 2 weeks of life[28]. This observation coupled with our previous report, which shows little association of Day 1 inflammatory protein elevations with 2-year cognitive outcomes[14], suggests that in addition to exposures that initiate an inflammatory process in utero, postnatal exposures also might initiate inflammation that predicts later cognitive deficits. Such postnatal exposures could injure the brain directly and also could constitute a second hit to an already sensitized central nervous system.[29, 30] Postnatal events that can contribute to risk over the first 4 weeks of life include lung inflammation, necrotizing entrerocolitis, derangements of blood pressure and oxygen exchange, acidosis, and bacteremia, fungemia, and/or sepsis.[31–33]

The mechanisms by which inflammation contributes to neurologic dysfunction are not yet known, but could include microglial activation, enhanced expression of cyclooxygenase 2, death of immature oligodendrocyte, excitotoxic-glutamatergic-mediated injury, disruptions to neuronal migration and survival, and impaired synaptogenesis[34]. The mechanisms may be more indirect, including the initiation of epigenetic-mediated changes that contribute to neuronal death, impededence production of neuroprotective proteins, and/or interference with neuroplasticity mechanisms.[29, 35–39] In addition, it is possible that disturbance of the infant's systemic immune-inflammatory balance may affect the establishment of a healthy microbiome at birth with consequent microbiota-mediated harmful effects on the brain.[40, 41]

Aside from the ELGAN Study, only two studies have assessed relationships between inflammation-related proteins[42, 43] in neonatal blood and subsequent developmental outcomes; one involved infants born at less than 1 kilogram[42], and the other 67 infants born at less than 32 weeks gestation.[43] Both studies associated TNF-alpha with poor cognitive outcomes. One also identified an elevation of IL-8[42] and the other an elevation of IL-6[43] as associated with decreased scores on one of the two Bayley Scales of Infant Development at 2 years of age. A large study that evaluated proteins in cord blood[44] did not identify any protein elevation as being associated with low Bayley MDI or PDI. Our study differed from these in that we examined school age outcomes, evaluated proteins over the course of the first month of life, and investigated sustained elevation of proteins.

Among adults, neurologic injury after cardiac arrest or severe head trauma is associated with elevations of many inflammatory markers, most elevations, which resolve within 12 hours.[45] Persisting IL-8 (and E-selectin) elevations, much as in our study, however, are associated with greater degrees of neurologic injury.[46] The elevations of inflammation-related proteins early in life that are associated with later cognitive impairment appear to persist for weeks. Although inflammation also might be a consequence of brain injury, evidence suggests that the process of inflammation resolution promotes feedback-loops between the immune system and the brain damage,[47] leading to augmented brain damage and increased neurologic dysfunction.[48]

The persistence of risk through the first 4 weeks of life and the limited risk associated with protein elevation on the first day of life imply a later and broader window of vulnerability than previously recognized, when specific therapeutic interventions targeting inflammatory processes might effectively decrease the risk of adverse outcomes. Therapies that can modulate inflammation, such as hypothermia[49], erythropoietin[50], and melatonin[50–52] show promise as strategies to improve brain-related outcomes in newborns. Inflammation[53–55], developmental regulation of immunity[56], immune response to infections[57], and stress-induced immune dysregulation[58] may be influenced by epigenetic alterations that activate the expression of pro-inflammatory cytokines. Pharmacologic agents that target epigenetic processes (e.g. histone deacteylase inhibitors[59–61] and folate, a one-carbon donor for DNA methlhylation[62]) show promise as therapies for dampening inflammation or its effects in the postnatal period.

Our data suggest that IL-8 and ICAM-1 are important risk modulators for cognitive outcome. IL-8, a chemokine, previously has been implicated as a marker of adverse neurologic outcomes in preterm newborns[63, 64] and in adults following cardiac arrest[65]. IL-8 plays a role in acute inflammation by recruiting and activating neutrophils[66] and specifically has been associated with increased risk of psychomotor development index < 70[42]. ICAM-1 is an adhesion molecule and is thought to play a crucial role in the pathogenesis of inflammation. Simvastatin reduces expression of ICAM-1 and has been associated with improved outcomes in a rat model of traumatic brain injury[67]. Elevations of ICAM-1 in humans with stroke have been associated with more severe neurologic deficits[68, 69]. Both IL-8 and ICAM-1 also specifically were elevated in our cohort of infants with cerebral white matter injury and/or intraventricular hemorrhage on early head ultrasound studies[27, 70], and in children at age 2 years with microcephaly[26], abnormal cognition[14], attentional disorders[71], and cerebral palsy[25].

The association of later adverse outcomes with the presence of early-life circulating inflammatory proteins probably is modulated by other factors associated with immaturity, including the availability of endogenous protective factors [72],[73],[74], such as oligotrophins. Heightened production and availability of oligotrophins could modulate the damaging effects associated with inflammation, for example, by enhancing cell development and/or survival [73]. In this report we focus on risk associated with cytokines and other effectors of acute inflammation, but a clearer understanding of the relationship of inflammation to neuronal risk and later cognitive consequences could derive from consideration of endogenous protectors as well.

Our study has several strengths. We included a large number of infants, collected our data prospectively, had only modest attrition across 10 years of follow-up, examiners at 2 and 10 years were not aware of the medical histories of the children they examined, and measured protein data are of high quality, with high content validity.[22, 23, 75]

A limitations of an observational study is that causation and association of study findings cannot be distinguished. Although we sampled a wide range of inflammation-associated proteins, including specific proteins known to be associated with neurologic damage, we did not evaluate all known inflammation-associated proteins. We selected proteins on the basis

of likely involvement in the fetal/neonatal inflammatory response and the accuracy with which they could be measured reliably in whole blood spots using the Meso Scale Discovery multiplex platform. It is likely that the 532 children who underwent late protein assessments were more ill than those who no longer required blood samples. Even though this probably does not bias associations, it could limit the degree to which these associations can be generalized to healthier preterm infants. Finally, rather than report absolute protein concentration values, we used a distribution-based definition of protein elevation according to gestational age, postnatal day, and the interval between processing blood samples, because normal values are not known and values appear to be influenced by these factors.

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Abbreviations

ELGAN	Extremely low gestational age newborn
NICU	Neonatal intensive care unit
BSID-II	Bayley Scales of Infant Development - Second Edition
MDI	mental development index of the BSID-II
PDI	psychomotor development index of the BSID-II
CRP	C-Reactive Protein
E-sel	E-selectin
EPO	Erythropoietin
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
IL-8	Interleukin-8
ICAM-1	Intercellular Adhesion Molecule −1
ICAM-3	Intercellular Adhesion Molecule −3
IGFBP-1	Insulin-like growth factor binding protein-1

I-TAC	Interferon-inducible T cell alpha-chemoattractant
MMP-9	Matrix Metalloproteinase-9
MCP-1	Monocyte chemotactic protein-1
MCP-4	Monocyte chemotactic protein-4
MIP-1 beta	Macrophage inflammatory protein-1beta
MMP-1	Metalloproteinase-1
MMP-9	Metalloproteinase-9
MPO	Myeloperoxidase
RANTES	Regulated upon Activation, Normal T-cell Expressed, and Secreted
SAA	Serum Amyloid A
TNF-α	Tumor Necrosis Factor-α
TNF-R2	Tumor Necrosis Factor Receptor-1
TNF-R1	Tumor Necrosis Factor Receptor-2
TSH	thyroid-stimulating hormone
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular Endothelial Growth Factor Receptor-1
VEGF-R1	Vascular Endothelial Growth Factor Receptor-2

Appendix

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Table 1

Percent of children with cognitive impairment by mother and child characteristics, n=812.

			DAS IQ Z-Score[1]		Impairment level by LPA		
		N	≤ −2 %	> −2, ≤ −1 %	Severe %	Moderate %	Low normal %
Maternal characteristics							
Racial identity	White	512	10	16	5	12	40
	Black	208	25	28	13	26	45
	Other	90	23	16	9	21	38
Hispanic	Yes	80	23	24	14	16	49
	No	731	15	18	7	17	40
Age, years	< 21	105	17	28	9	26	42
	21–35	546	16	18	8	16	42
	> 35	161	12	15	7	13	38
Education, years	≤ 12	334	22	24	11	23	46
	> 12, < 16	190	15	22	8	17	42
	≥ 16	288	8	11	3	9	35
Single marital status	Yes	329	19	27	10	23	46
	No	483	13	13	7	12	37
Public insurance	Yes	286	23	26	12	25	44
	No	526	11	15	6	12	40
Newborn characteristics							
Sex	Male	413	20	18	10	18	39
	Female	399	11	19	6	15	44
Gestational age, weeks	23–24	173	26	20	16	21	40
	25–26	366	15	21	7	17	42
	27	273	9	15	4	13	41
Birth weight, grams	≤ 750	306	24	22	14	23	41
	751–1000	347	11	18	4	14	44
	> 1000	159	8	14	5	11	35

				DAS IQ Z-Score[1]		Impairment level by LPA		
		N	≤ −2 %	> −2, ≤ −1 %	Severe %	Moderate %	Low normal %
Birth weight Z-score	< −2	49	19	23	8	24	41
	≥ −2, < −1	106	20	24	8	24	41
	≥ −1	657	15	18	8	15	41
Bayley Scales at age 2 years							
Mental Development Index	< 55	109	56	16	38	29	26
	55–69	80	33	25	11	29	46
	≥ 70	564	5	18	1	12	44
Psychomotor Development Index	< 55	109	52	18	38	24	28
	55–69	115	18	24	4	24	44
	≥ 70	529	7	17	2	13	43

[1] n=809 for DAS IQ, 3 children completed only the verbal or nonverbal IQ, so an average could not be calculated

Table 2

Adjusted odds ratios[1] and 95% confidence intervals for impaired IQ[2] at age 10 years for those with elevated inflammatory protein concentrations[3] on 2 of the first 3 postnatal measurements (n=809)[4].

	N	DAS IQ Z-score	
	Elevated/ Not Elevated	≤ −2 (n=125)	> −2, ≤ −1 (n=153)
CRP	155 / 654	**2.0 (1.2, 3.2)**	1.4 (0.9, 2.2)
SAA	133 / 676	1.6 (0.97, 2.7)	1.5 (0.9, 2.4)
MPO	142 / 667	1.0 (0.6, 1.6)	1.1 (0.7, 1.7)
IL-1β	132 / 677	1.6 (0.98, 2.7)	1.1 (0.6, 1.8)
IL-6	131 / 678	2.0 (1.2, 3.3)	1.2 (0.8, 2.0)
IL-6R	161 / 648	0.7 (0.4, 1.2)	1.1 (0.7, 1.7)
TNF-α	154 / 655	**2.0 (1.2, 3.2)**	**1.6 (1.00, 2.4)**
TNF-R2	138 / 671	1.3 (0.8, 2.1)	1.0 (0.6, 1.7)
IL-8 (CXCL8)	136 / 673	**2.3 (1.4, 3.8)**	**1.6 (1.02, 2.6)**
RANTES (CCL5)	149 / 660	0.7 (0.4, 1.2)	0.7 (0.4, 1.2)
ICAM-1 (CD54)	152 / 687	**2.3 (1.4, 3.7)**	1.5 (0.9, 2.3)
MMP-9	122 / 687	0.7 (0.4, 1.3)	0.9 (0.6, 1.6)
VEGF	164 / 645	0.7 (0.4, 1.1)	0.9 (0.6, 1.4)
VEGF-R2	164 / 645	1.1 (0.7, 1.8)	1.2 (0.8, 1.9)
TSH	164 / 645	1.2 (0.7, 1.9)	1.0 (0.6, 1.5)
EPO	138 / 671	**2.2 (1.3, 3.5)**	1.4 (0.9, 2.3)

[1] Odds ratios comparing indicated IQ group to those with IQ Z-score above −1, adjusting for public insurance and child sex through multinomial logistic regression

[2] IQ z-score based on average of the DASII verbal and non-verbal IQ scores

[3] Inflammatory protein concentration in the top quartile controlling for gestational age and day of measurement

[4] 3 children completed only the verbal or nonverbal IQ, so an average could not be calculated

Table 3

Adjusted odds ratios[1] and 95% confidence intervals of overall cognitive impairment based on Latent Profile Analysis at age 10 years for those with elevated concentrations of inflammatory proteins[2] on 2 of the first 3 postnatal measurements (early period) and on both the 21st and 28th postnatal day measurements (controlling for early protein level, late period).

	Early* (n=812)				**Late in light of early** (n=532)**			
	Impairment level by LPA							
	N Elevated	**Severe (n=64)**	**Moderate (n=135)**	**Low normal (n=333)**	**N Elevated**	**Severe (n=45)**	**Moderate (n=98)**	**Low normal (n=221)**
CRP	156	**2.1 (1.05, 4.2)**	**2.4 (1.4, 4.1)**	**1.8 (1.1, 2.8)**	62	**4.0 (1.5, 10)**	2.0 (0.8, 4.6)	1.7 (0.8, 3.5)
SAA	133	**2.1 (1.01, 4.2)**	**2.0 (1.1, 3.5)**	1.6 (0.98, 2.5)	48	2.6 (0.9, 7.7)	2.0 (0.9, 5.1)	1.2 (0.6, 2.7)
MPO	143	1.1 (0.6, 2.3)	0.9 (0.5, 1.6)	1.0 (0.7, 1.5)	64	0.6 (0.2, 2.2)	1.0 (0.5, 2.3)	1.2 (0.7, 2.2)
IL-1β	132	1.9 (0.9, 3.8)	1.5 (0.8, 2.6)	1.2 (0.8, 1.9)	65	0.5 (0.1, 2.0)	1.9 (0.9, 4.0)	1.1 (0.6, 2.0)
IL-6	131	**2.5 (1.3, 5.0)**	**2.0 (1.2, 3.6)**	1.3 (0.8, 2.1)	62	2.0 (0.8, 5.5)	1.4 (0.6, 3.1)	1.2 (0.6, 2.4)
IL-6R	163	0.5 (0.2, 1.2)	1.1 (0.6, 1.8)	1.1 (0.7, 1.6)	67	1.1 (0.4, 3.1)	0.6 (0.3, 1.5)	1.0 (0.6, 1.9)
TNF-α	154	**2.2 (1.1, 4.3)**	**2.8 (1.6, 4.6)**	1.4 (0.9, 2.2)	89	0.7 (0.2, 2.0)	1.7 (0.9, 3.3)	1.1 (0.6, 1.9)
TNF-R2	138	1.6 (0.8, 3.2)	1.2 (0.7, 2.1)	1.1 (0.7, 1.8)	67	2.2 (0.8, 5.6)	1.6 (0.7, 3.5)	1.4 (0.7, 2.7)
IL-8 (CXCL8)	136	**2.8 (1.4, 5.5)**	**2.2 (1.3, 3.9)**	1.4 (0.9, 2.3)	80	**5.0 (1.9, 13)**	**6.3 (2.8, 14)**	**2.4 (1.1, 5.1)**
RANTES (CCL5)	151	0.6 (0.3, 1.3)	0.8 (0.4, 1.3)	0.7 (0.5, 1.1)	65	1.3 (0.5, 3.3)	0.9 (0.4, 2.0)	0.8 (0.4, 1.4)
ICAM-1 (CD54)	153	**3.1 (1.6, 6.0)**	**2.4 (1.4, 4.2)**	1.5 (0.9, 2.3)	87	**6.5 (2.6, 16)**	**2.9 (1.3, 6.4)**	**2.7 (1.4, 5.4)**
MMP-9	124	0.5 (0.2, 1.3)	1.1 (0.6, 2.0)	1.2 (0.8, 1.9)	53	0.9 (0.3, 2.9)	1.0 (0.4, 2.3)	0.9 (0.5, 1.8)
VEGF	165	0.7 (0.3, 1.4)	0.7 (0.4, 1.2)	0.8 (0.6, 1.2)	68	0.8 (0.3, 2.2)	0.9 (0.4, 2.0)	0.6 (0.3, 1.2)
VEGF-R2	161	1.2 (0.6, 2.4)	1.2 (0.7, 2.0)	1.1 (0.8, 1.7)	71	**3.2 (1.2, 8.3)**	**2.4 (1.1, 5.4)**	**2.0 (1.01, 4.1)**
TSH	165	1.4 (0.8, 2.8)	1.0 (0.6, 1.7)	1.1 (0.7, 1.6)	73	**3.1 (1.3, 7.3)**	1.4 (0.6, 3.0)	1.1 (0.6, 2.1)
EPO	138	**3.6 (1.8, 7.1)**	**2.3 (1.3, 4.1)**	1.8 (1.1, 3.0	65	1.0 (0.3, 3.0)	1.4 (0.6, 3.1)	1.3 (0.7, 2.5)

[1] Odds ratio comparing the indicated category to those with normal overall cognitive function, controlling for public insurance at birth and child sex

[2] * Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

** Information added by the late concentrations (protein in the highest quartile on both day 21 and 28.

Table 4

Adjusted odds[1] ratios and 95% confidence intervals for measures of cognitive impairment at age 10 years associated with 4+ [2] or 2–3 elevated proteins on 2 of postnatal days 1, 7, and 14 (early period), on both postnatal days 21 and 28 controlling for early elevation (late period), and with both early and late 4+ elevations, or one with 4+ and the other having 2–3 elevations (4+ & 2–3+).

		Early[3] (n=812)		Late in light of early[3] (n=532)		Both early and late[4] (n=532)	
		4+ (n=130)	2–3 (n=179)	4+ (n=47)	2–3 (n=115)	4+ (n=16)	4+ & 2–3+ (n=270)
DAS IQ[5] Z-score	≤ −2	**2.4 (1.4, 4.0)**	1.3 (0.8, 2.1)	**3.2 (1.5, 6.9)**	**2.2 (1.2, 3.9)**	**5.9 (1.7, 21)**	**2.1 (1.3, 3.5)**
	> −2, ≤ −1	1.5 (0.9, 2.5)	1.3 (0.9, 2.5)	1.1 (0.5, 2.7)	1.3 (0.8, 2.3)	2.3 (0.6, 8.8)	1.2 (0.8, 1.9)
LPA level of impairment	Severe	**2.3 (1.1, 4.9)**	1.1 (0.5, 2.3)	**4.8 (1.5, 16)**	1.5 (0.6, 3.5)	5.8 (0.7, 48)	**2.2 (1.1, 4.6)**
	Moderate	**2.8 (1.5, 5.0)**	**1.9 (1.1, 3.2)**	**5.1 (1.9, 14)**	1.7 (0.9, 3.3)	**9.3 (1.8, 50)**	**2.0 (1.2, 3.5)**
	Low normal	**1.7 (1.01, 2.7)**	1.1 (0.7, 1.6)	1.9 (0.8, 4.8)	1.0 (0.6, 1.7)	2.2 (0.4, 1.2)	1.3 (0.9, 2.9)

[1] Odds ratio comparing the indicated category to those without elevated proteins, controlling for public insurance at birth and child sex

[2] Number of elevated proteins among CRP, SAA, IL-1β, IL-6, TNF-α, IL-8, ICAM-1, MMP-9, and VEGF

[3] Referent protein group is < 2 elevations

[4] Referent protein group is early < 2 elevations and late < 2 elevations

[5] n=809 for DAS IQ, 3 children completed only the verbal or nonverbal IQ, so an average could not be calculated

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